The biology and therapeutic promise of small RNAS

Phillip Sharp

Koch Institute for Integrative Cancer Research

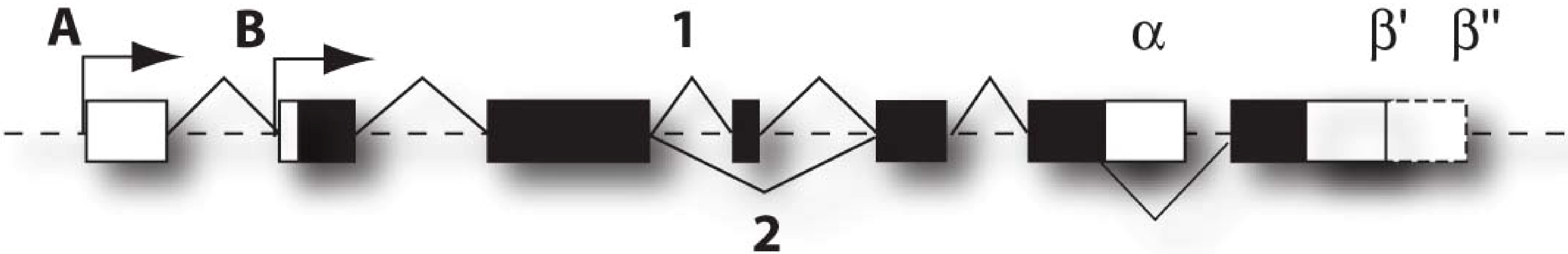
RNA in the transfer of information from DNA to protein

a) mRNA - message from DNA
b) t-RNA - intermediate between nucleic acid sequence and amino acid
c) Ribosomal RNA - components of decoding machine

Cellular environment can modify nature of mRNA in transfer of information from DNA to protein

a) Alternative RNA splicing
b) Alternative polyadenylation and RNA editing

EST evidence has demonstrated several modes of variation for transcripts coming from a single locus

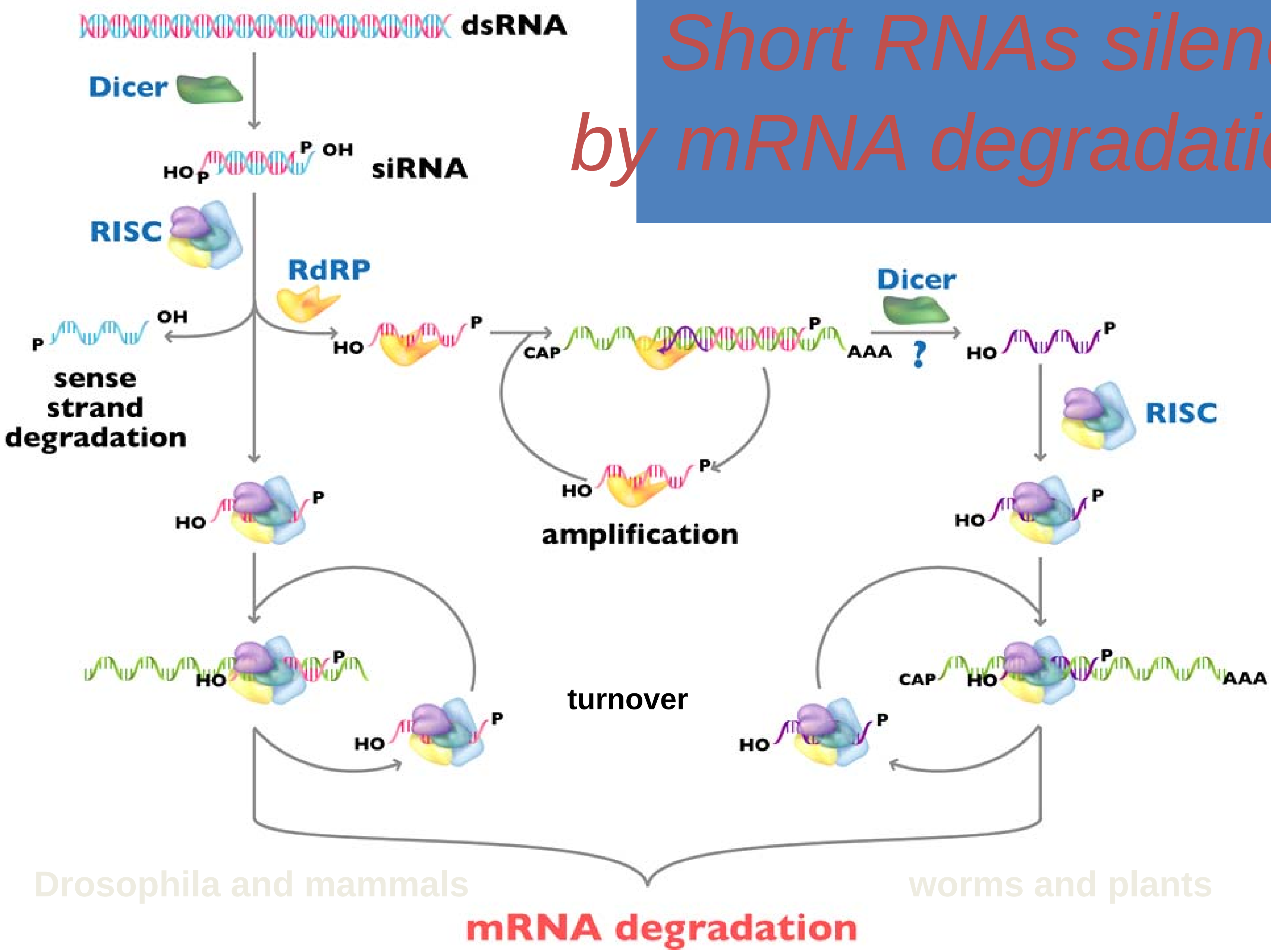


- Standard transcriptional activation
- Alternative promoter usage
- Exon inclusion/exclusion
- 3' UTR utilization
- All of the above

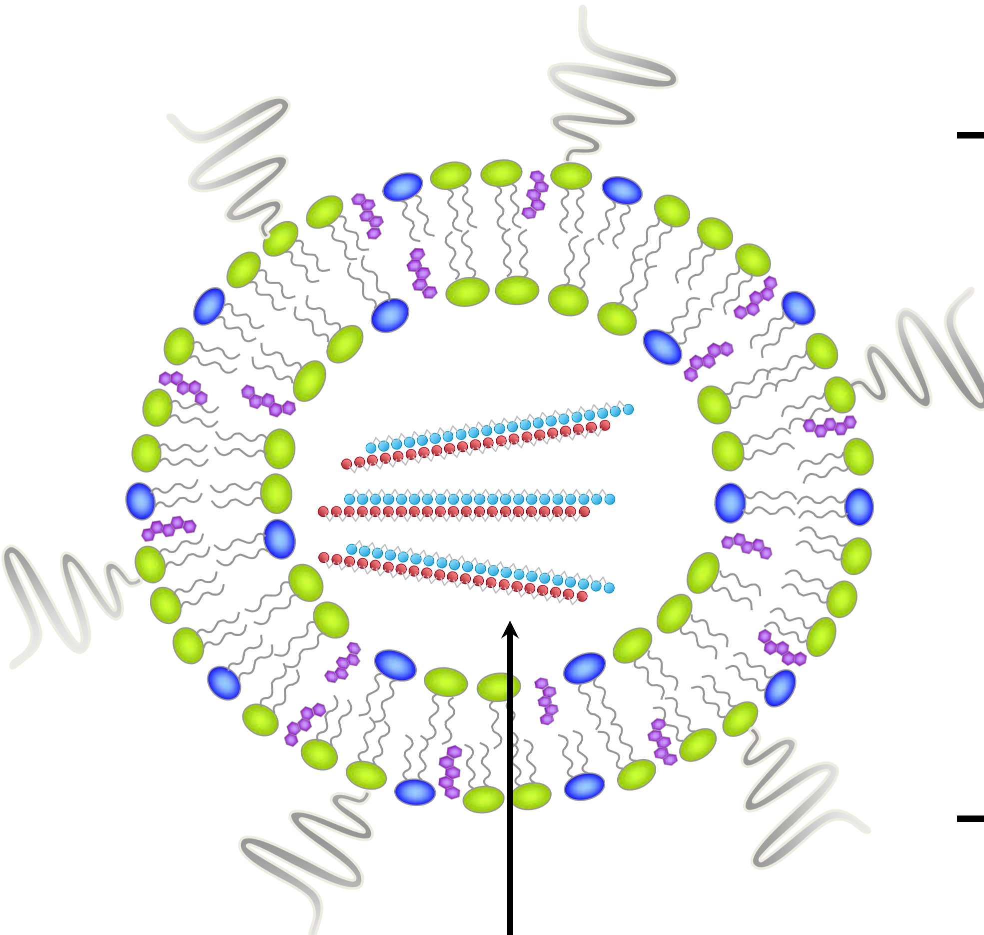
RNA interference (RNAi)

- Generated by dsRNA
- Silences genes at post-transcriptional stage by cleavage of mRNA
- Related process silences genes at translation
- Related process silences genes at transcriptional stage: transposable elements in genome

Short RNAs silence by mRNA degradation



Liposomal formulations for systemic RNAi

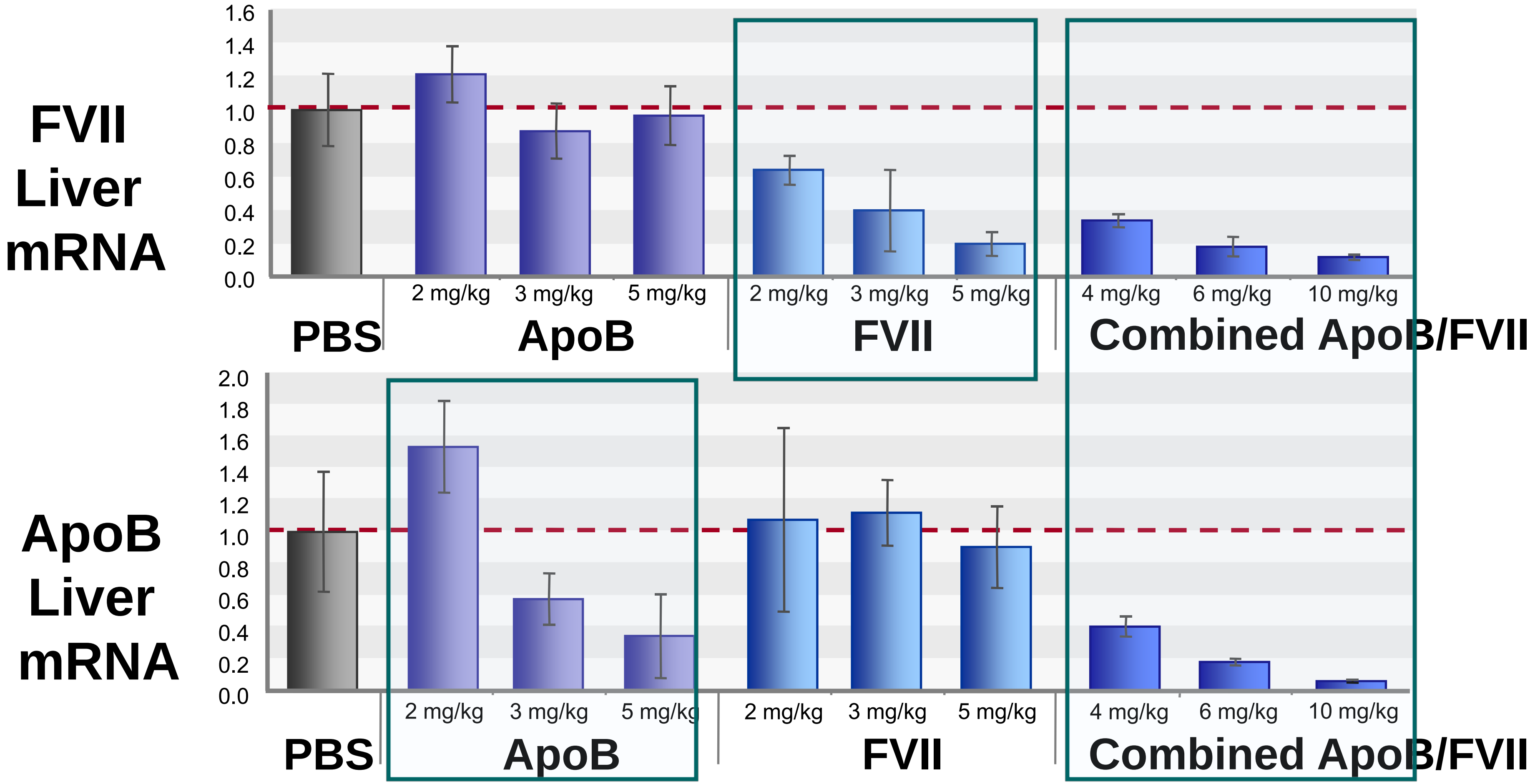


- Liposomes are organized lipid nanoparticles used in pharmaceutical drug delivery
  - Used in over 12 approved drugs
- Enables delivery of siRNAs into cells
  - Highly efficient for liver delivery

In vivo silencing of 2 distinct genes

Mouse

Delivery of 2 siRNAs in liposomal nanoparticles

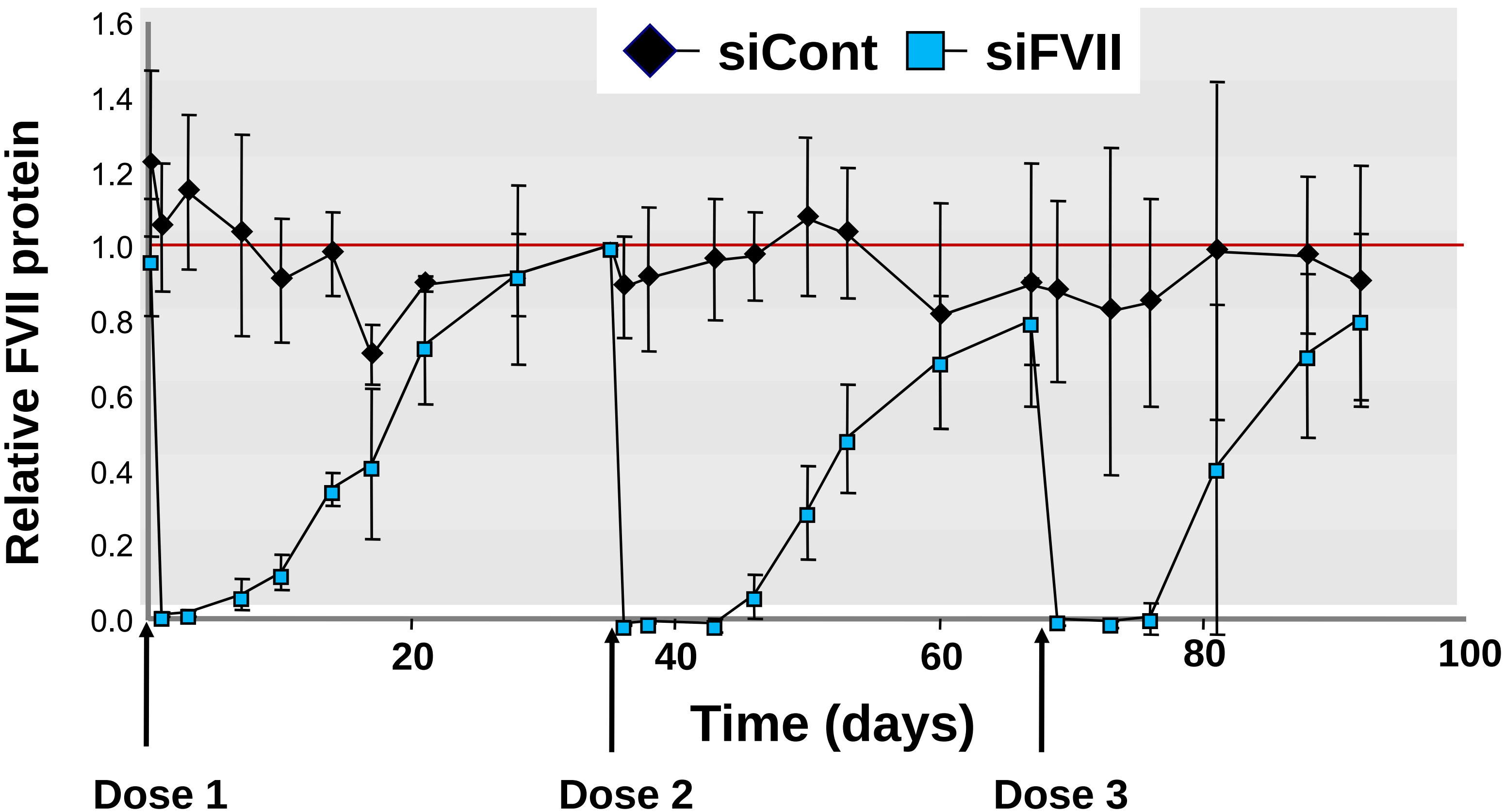


AACR Meeting, 2007

Repeat silencing of Factor VII

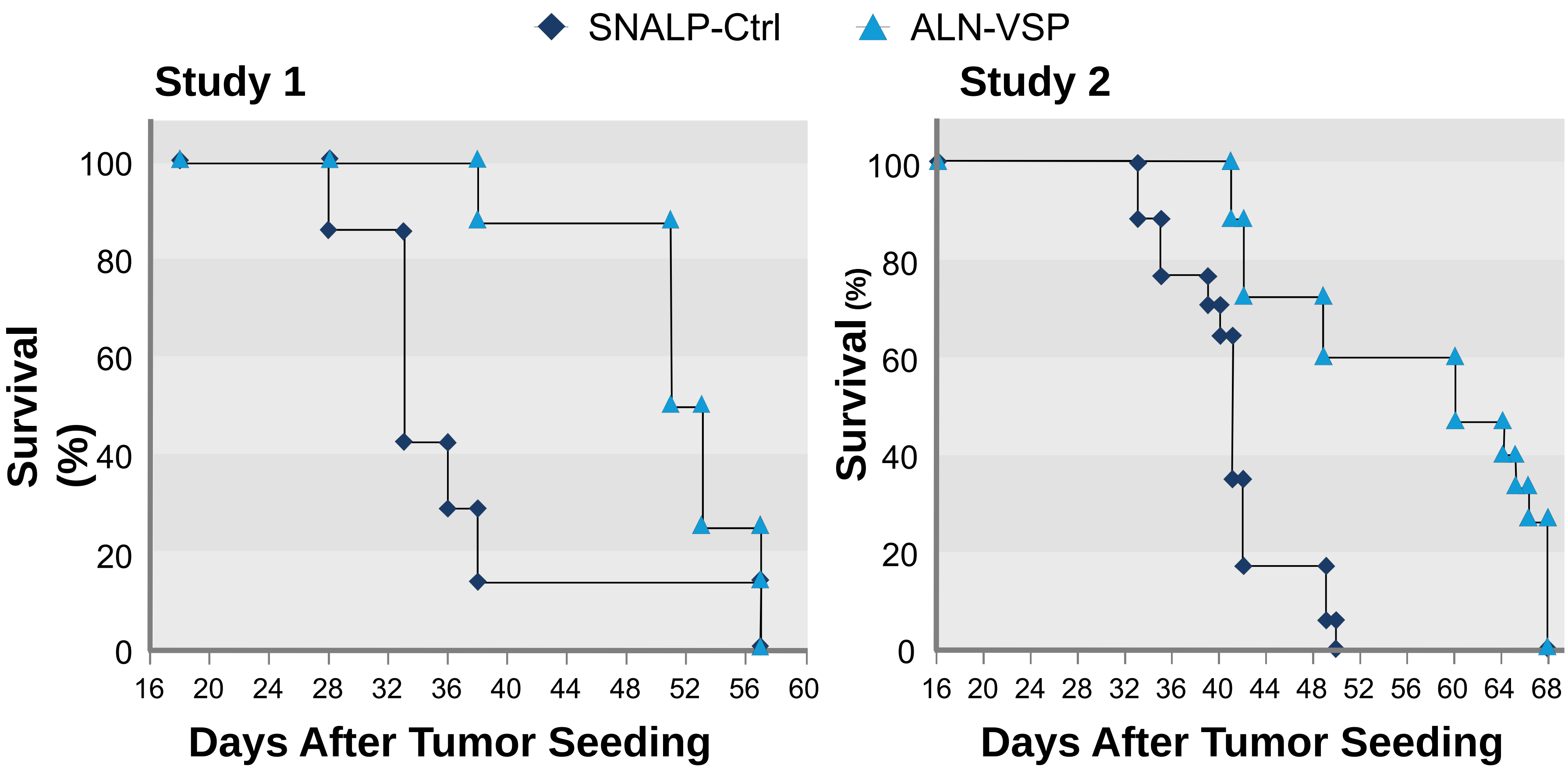
Rat model

Repeat dosing over 3 months is highly effective



Akinc *et al., Nature Biotechnology* (submitted)

Prolonged survival with ALN-VSP treatment



Orthotopic Tumor Models (Hep3B)

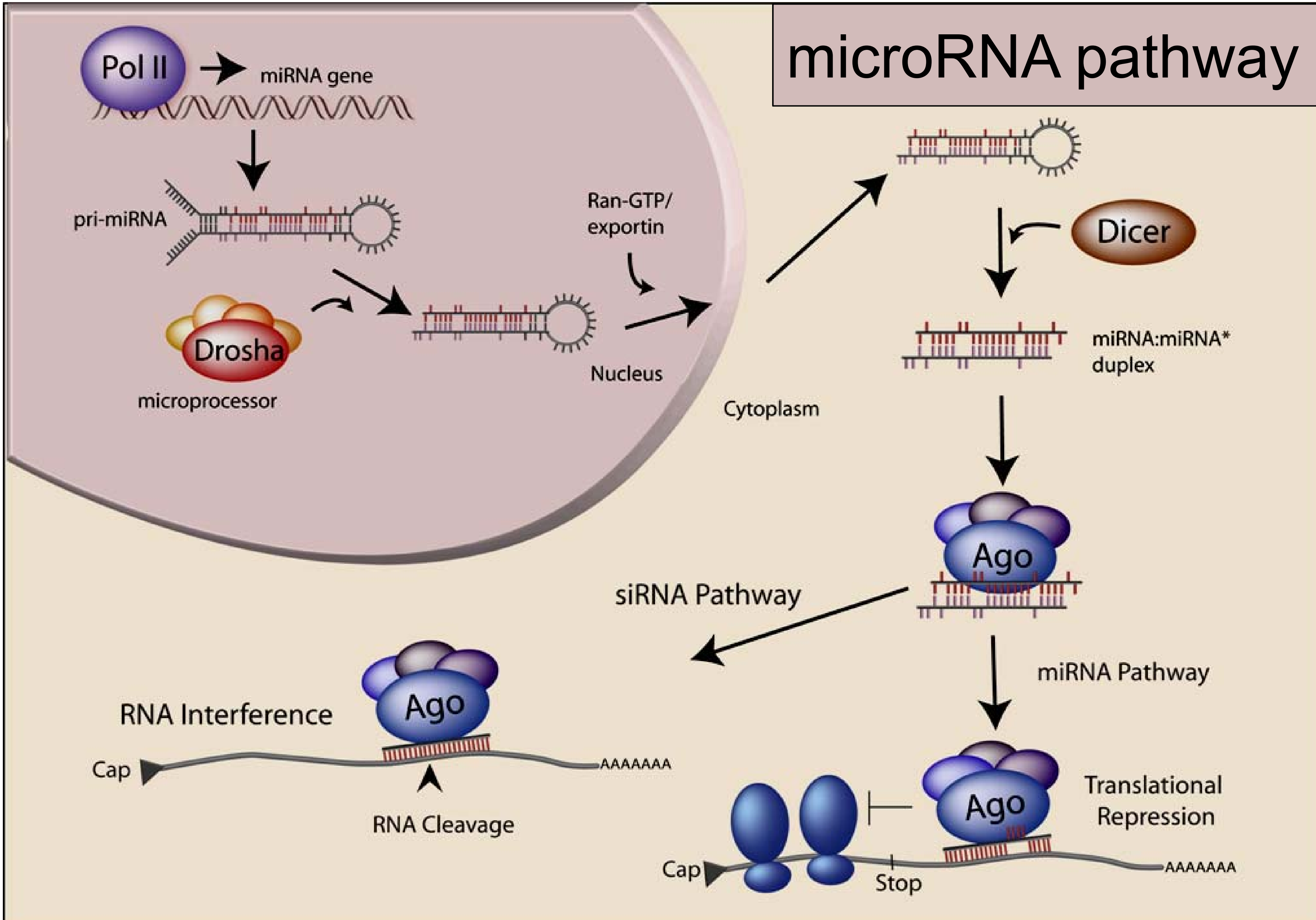
1. Treated 18 days post seeding; IV bolus injections of 4 mg/kg VSP or control siRNA 2x/wk for 3 wks
2. Treated 26 days post seeding; IV bolus injections of 4 mg/kg VSP or control siRNA 2x/wk for 3 wks

Keystone Symp: Ther. Mod. of RNA Using Oligos, Feb 2009

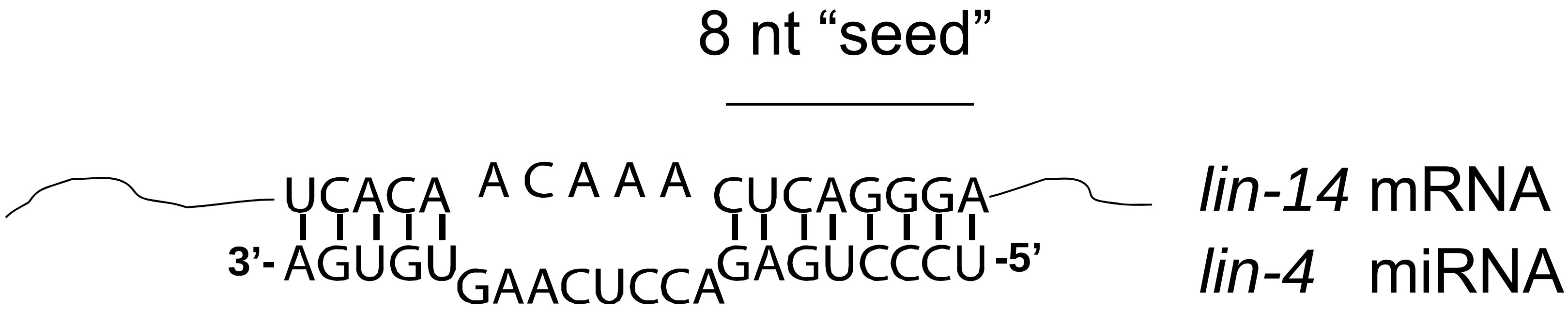
The discovery of 250 to 1000 new genes that encode microRNAs. These probably regulate 25-50% of all genes in vertebrates.

V. Ambros (Lee *et al.* 1993)
L. Ruvkun (Wightman *et al.* 1993)
T. Tuschl (Lagos-Quintana *et al.* 2001)
D. Bartel (Lau *et al.* 2001)
V. Ambros (Lee and Ambros, 2001)

microRNA pathway

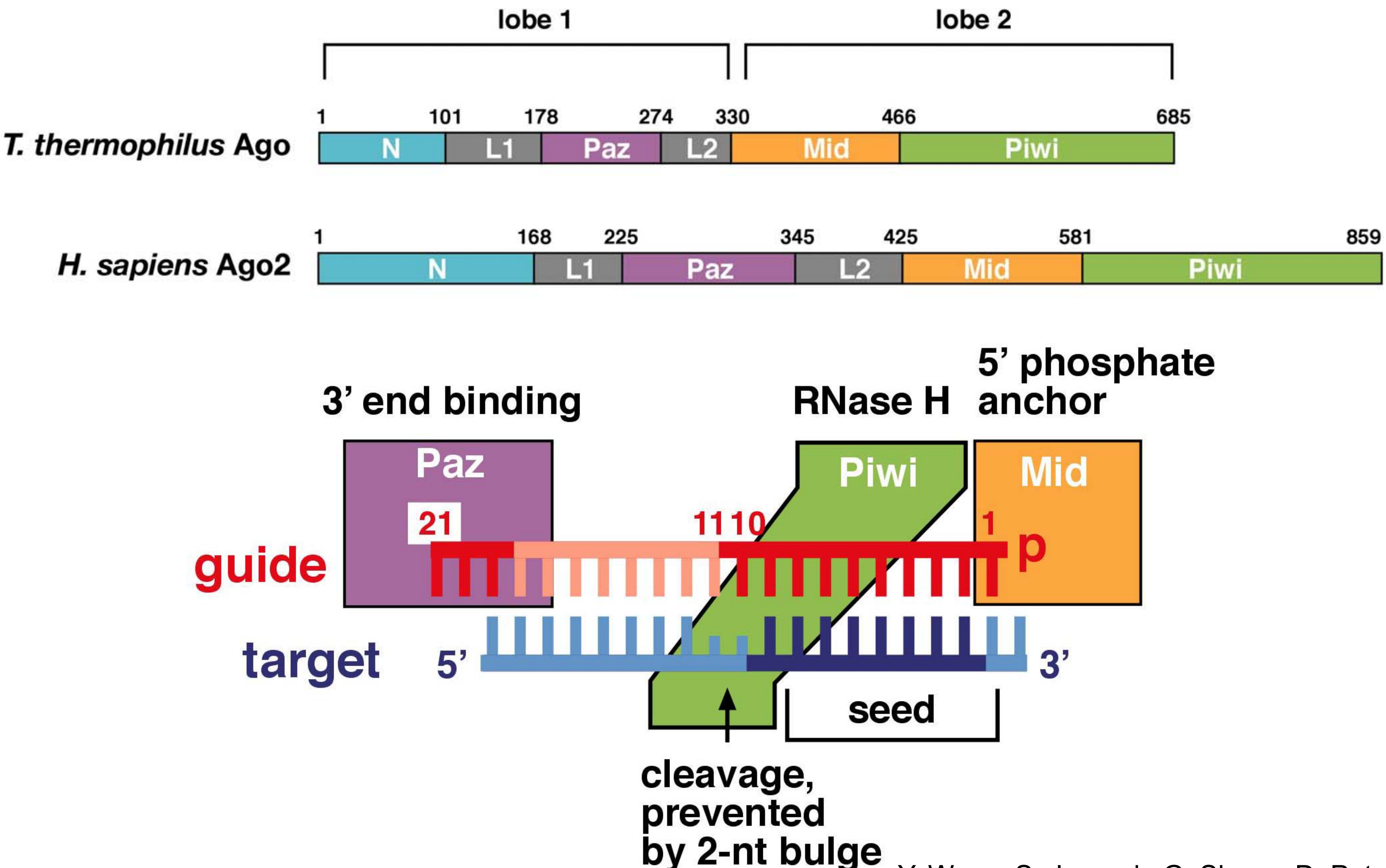


Proposed anatomy of miRNA/mRNA interactions



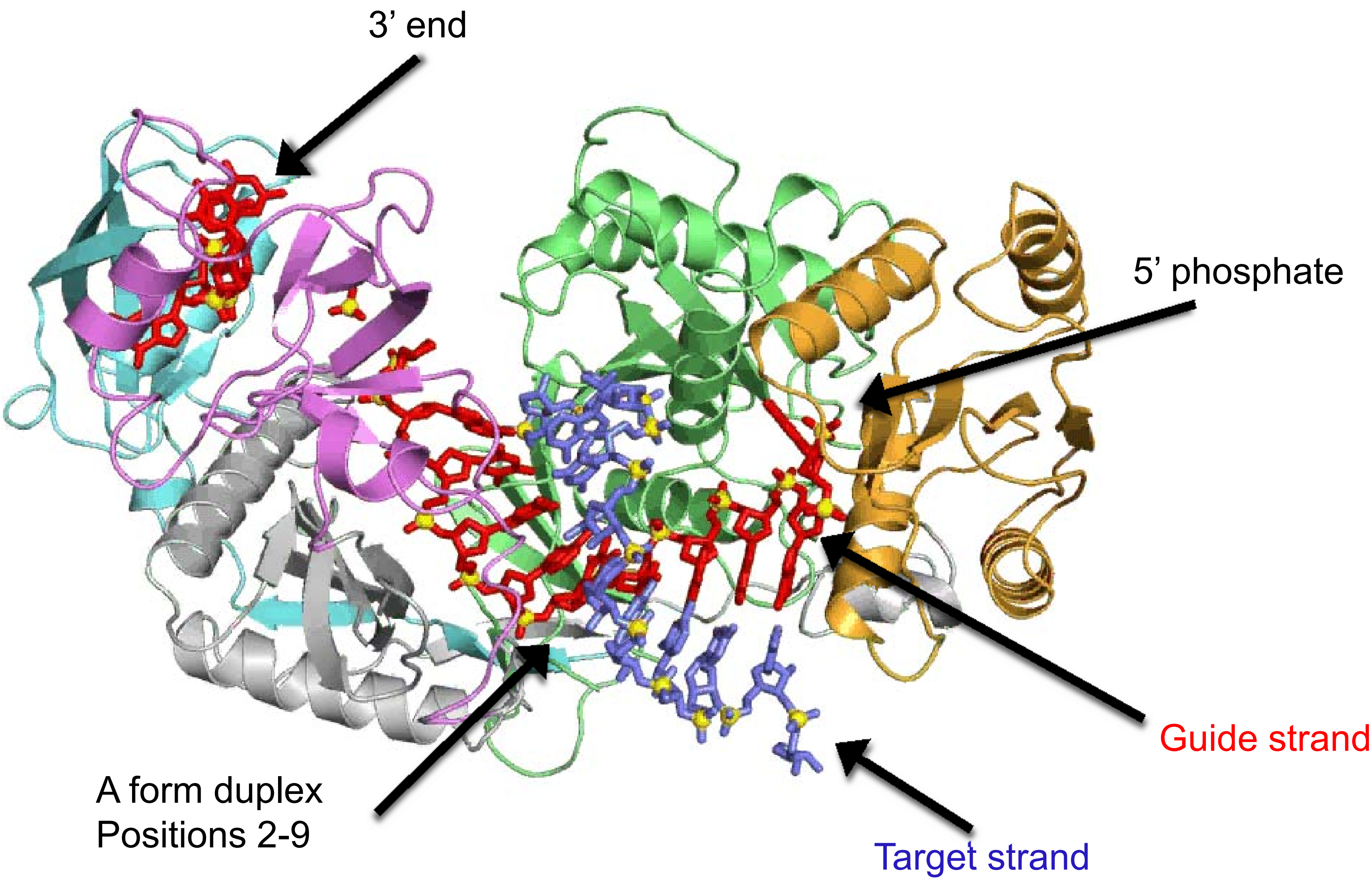
V. Ambros (Lee *et al.* 1993)
L. Ruvkun (Wightman *et al.* 1993)

Structural insights into mammalian Argonaute function from studies of bacterial Argonaute (D. Patel and T. Tuschl)



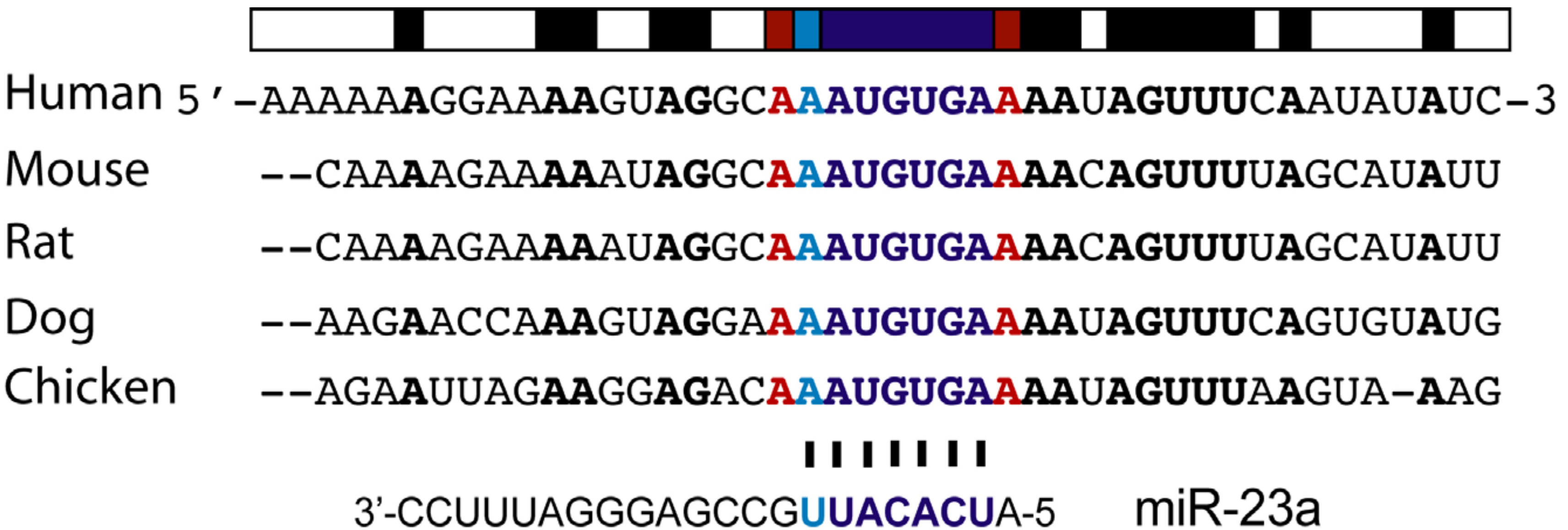
Y. Wang, S. Juranek, G. Sheng, D. Patel

T. th Ago-Guide DNA-Target RNA Ternary Complex



Y. Wang[1], S. Juranek[2], H. Li[1], G. Sheng[1], T. Tuschl[2]& D. J. Patel[1],*Nature in press*

Conserved seed matches in 3’ UTRs indicate extensive regulation by miRNAs



25-50% of all mRNAs interact with miRNAs

B.P. Lewis *et al.* 2005
Cell 120:15

Summary of target predictions for miRNA regulation in mammals

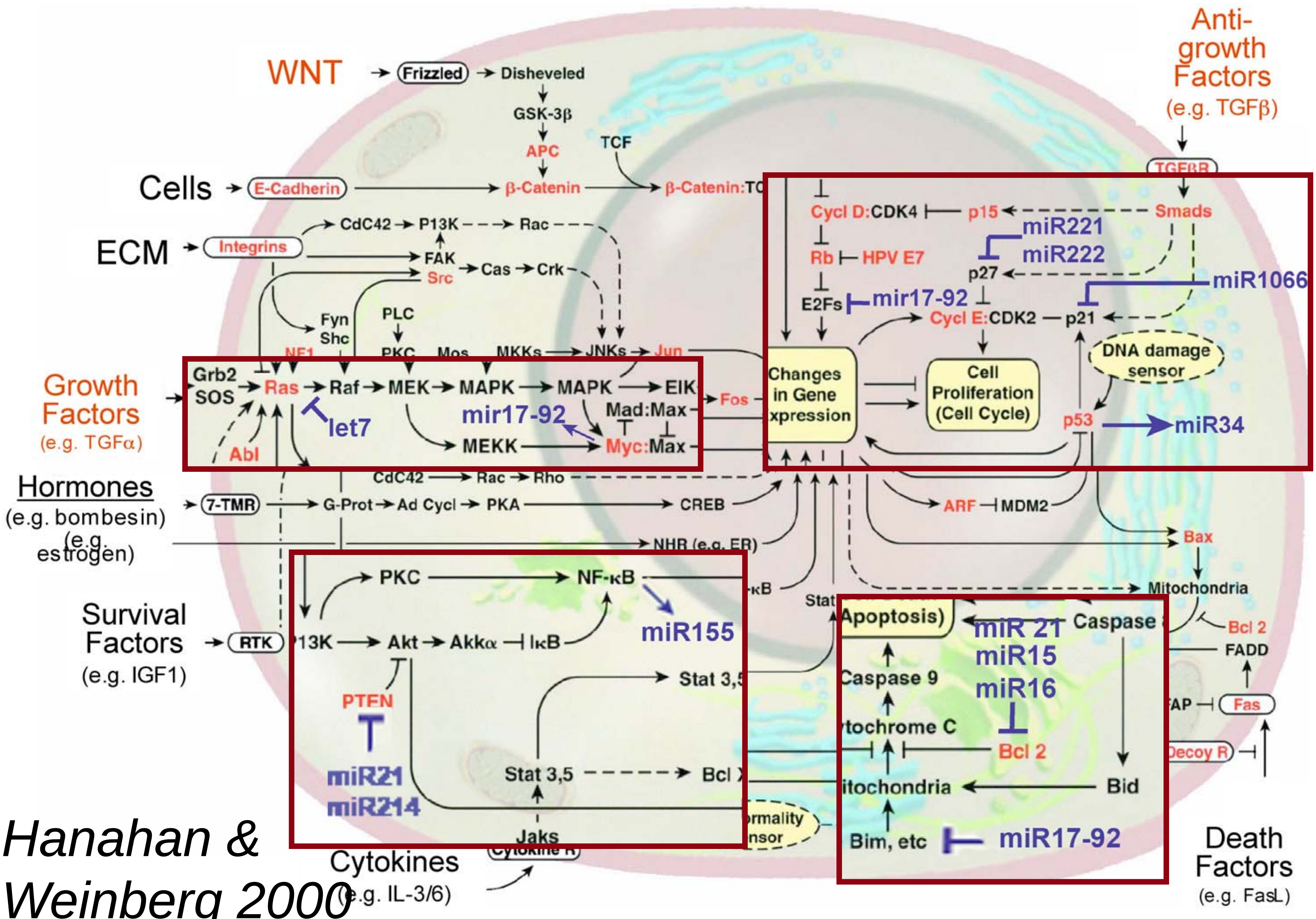
87 evolutionarily conserved “seed families” of miRNAs

Distribution of preferentially conserved target sites:

- 45% of genes - no sites in 3’ UTR
- 55% of genes - one or more target sites
  - Average is 4.2 sites per UTR and more than one family
  - Average 500 target mRNAs per family

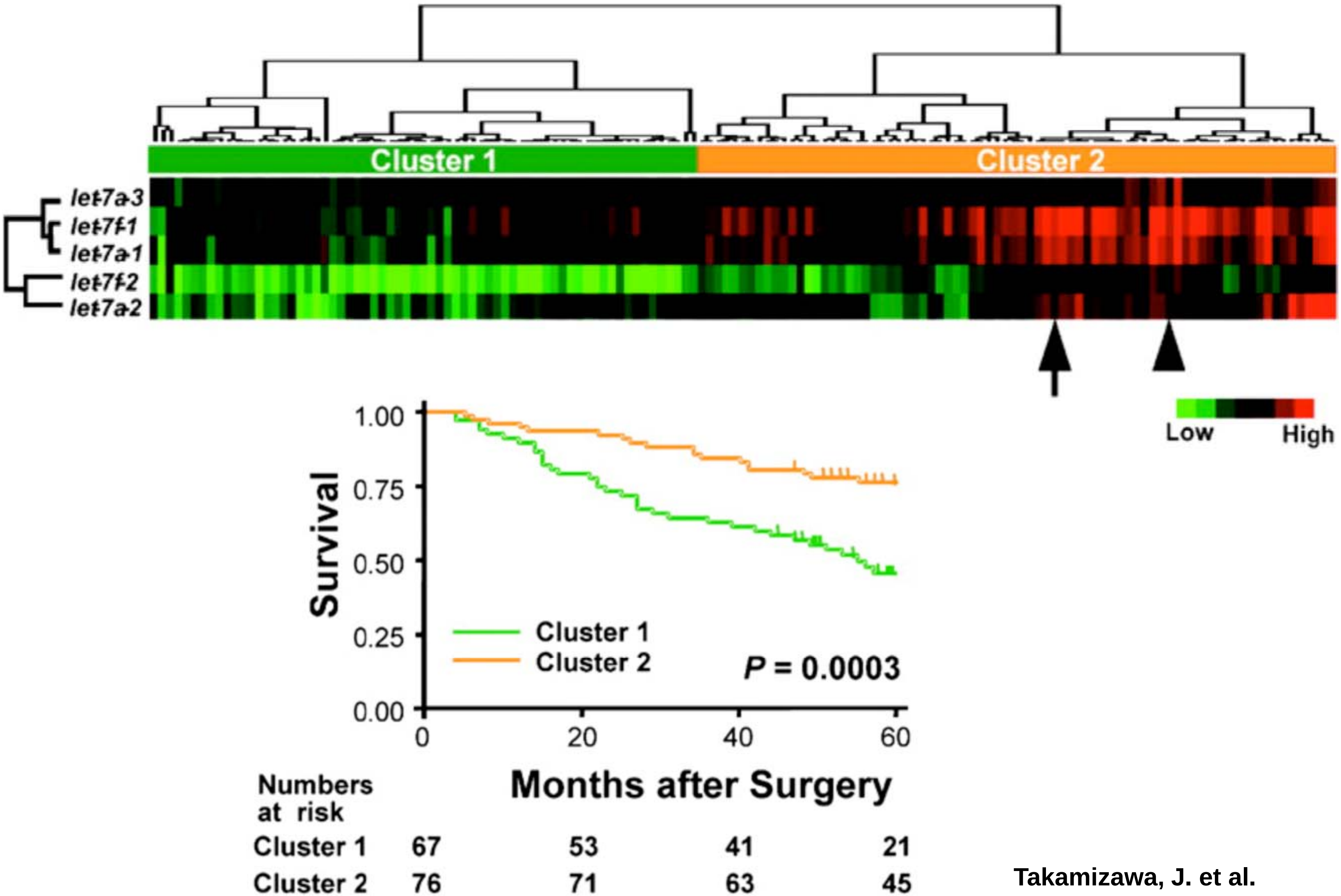
Source: David Bartel

microRNA regulation in cancer network



Hanahan & Weinberg 2000

let-7 is negatively regulated in non-small cell lung cancer (NSCLC)



Takamizawa, J. et al.
Cancer Res 2004;64:3753-3756

A role of microRNA regulation-Robustness

1) Stabilize quiescent and differentiated state
   - microRNA-let7 suppresses Ras, etc (growth) (*Frank Slack)*
   - Cell-type specific microRNAs suppresses genes expressed in other cellular states (*Lee Lim-05 and Stephen M. Cohen-05*)
   - Suppress cell-type specific genes
2) Control responses to changes in environment
   - Protection against abnormal cellular states (p53 and NF-κB) and stabilize cellular states from transient "stress"

Alternative cleavage and polyadenylation

Proliferating Cells Express mRNAs with Shortened 3′ Untranslated Regions and Fewer MicroRNA Target Sites

Rickard Sandberg,[1*†] Joel R. Neilson,[2*] Arup Sarma,[3]
Phillip A. Sharp,[1,2‡] Christopher B. Burge[1‡]

Science 20 June 2008:
Vol. 320. no. 5883, pp. 1643

Widespread Shortening of 3′UTRs by Alternative Cleavage and Polyadenylation Activates Oncogenes in Cancer Cells

Christine Mayr[1,2,3,4,*] and David P. Bartel[1,2,3,*]

Cell *138*, 673–684, August 21, 2009

Progressive lengthening of 3′ untranslated regions of mRNAs by alternative polyadenylation during mouse embryonic development

Zhe Ji[1], Ju Youn Lee[1], Zhenhua Pan[1], Bingjun Jiang, and Bin Tian[2]

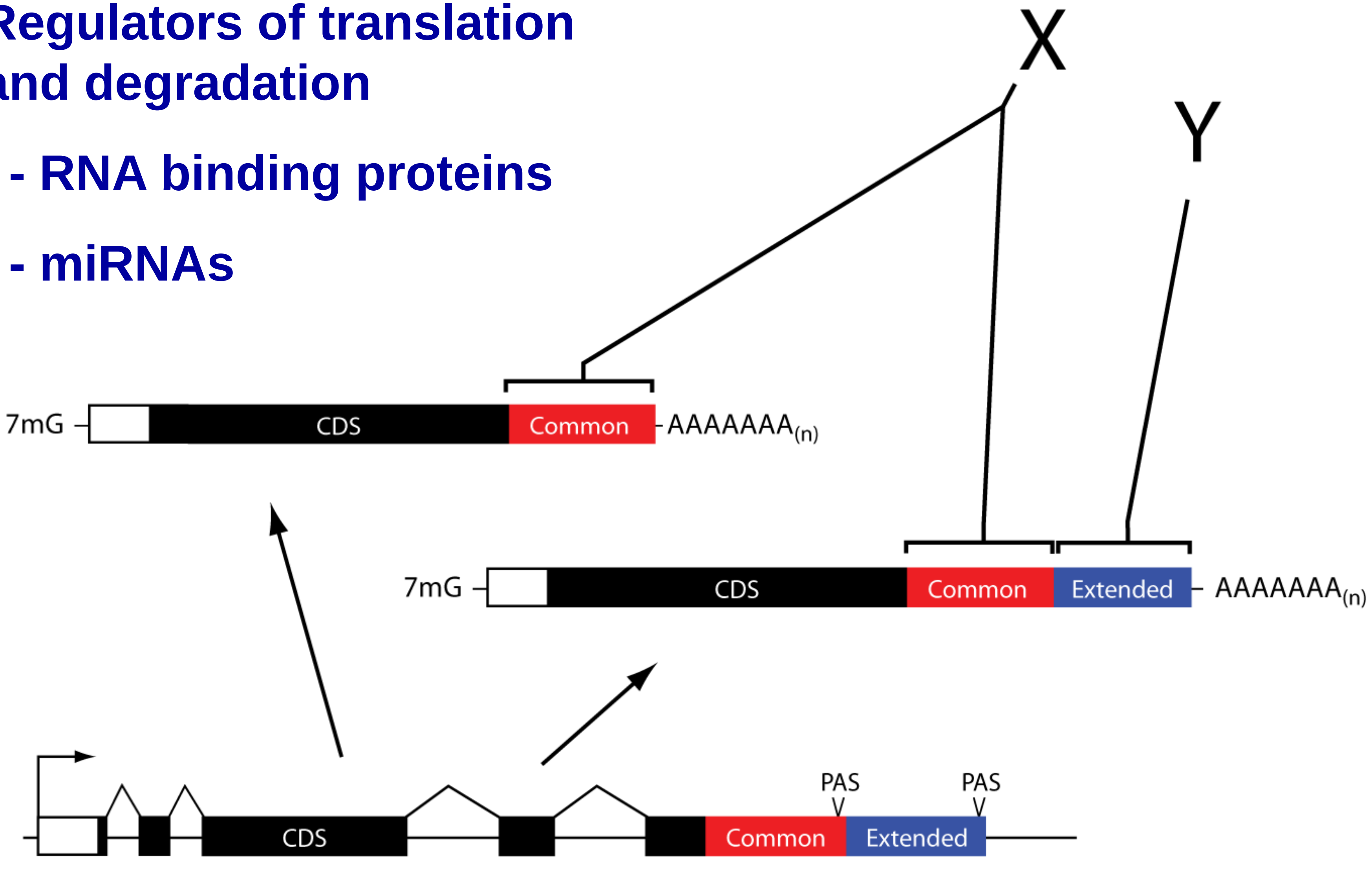
7028–7033 | PNAS | April 28, 2009 | vol. 106

Department of Biochemistry and Molecular Biology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103

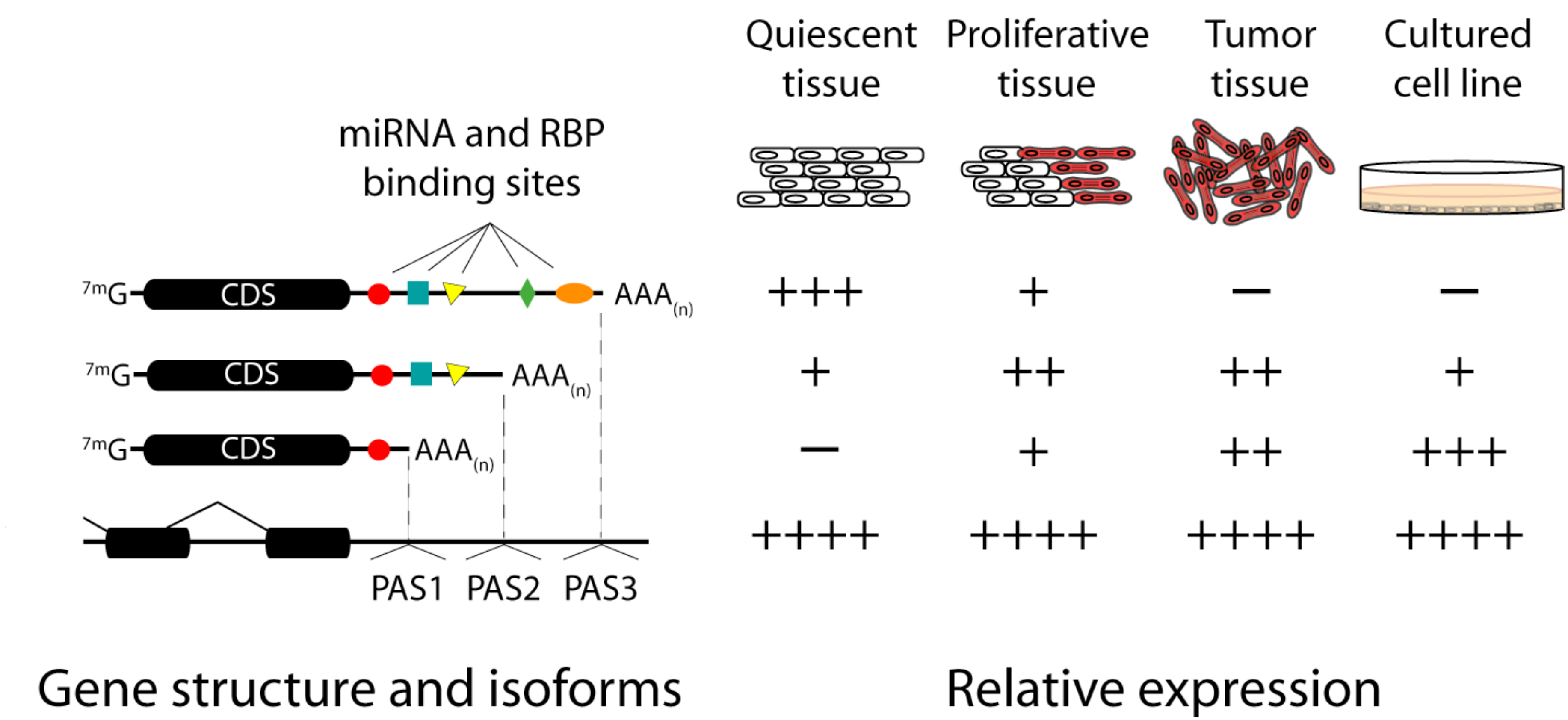
A model for evasion of post-transcriptional regulation

Regulators of translation and degradation

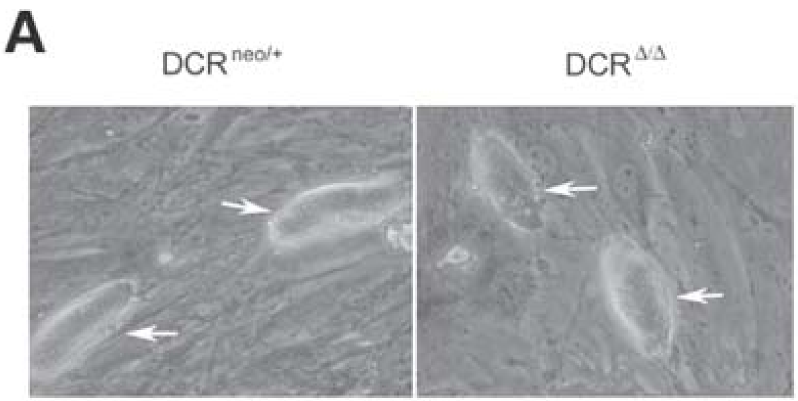
- **RNA binding proteins**
- **miRNAs**



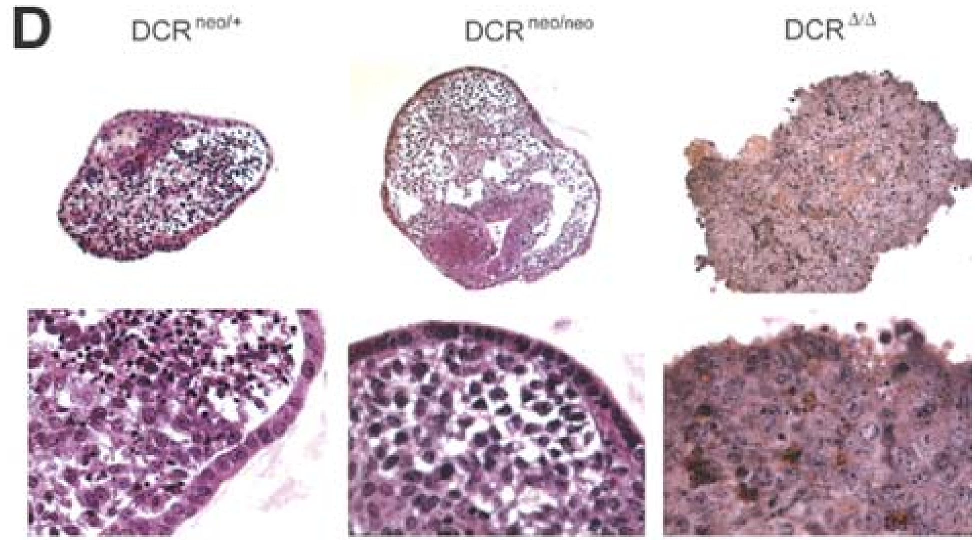
Post-transcriptional regulation critical for quiescent tissue



ES cells survive Dicer deletion, but are no longer pluripotent

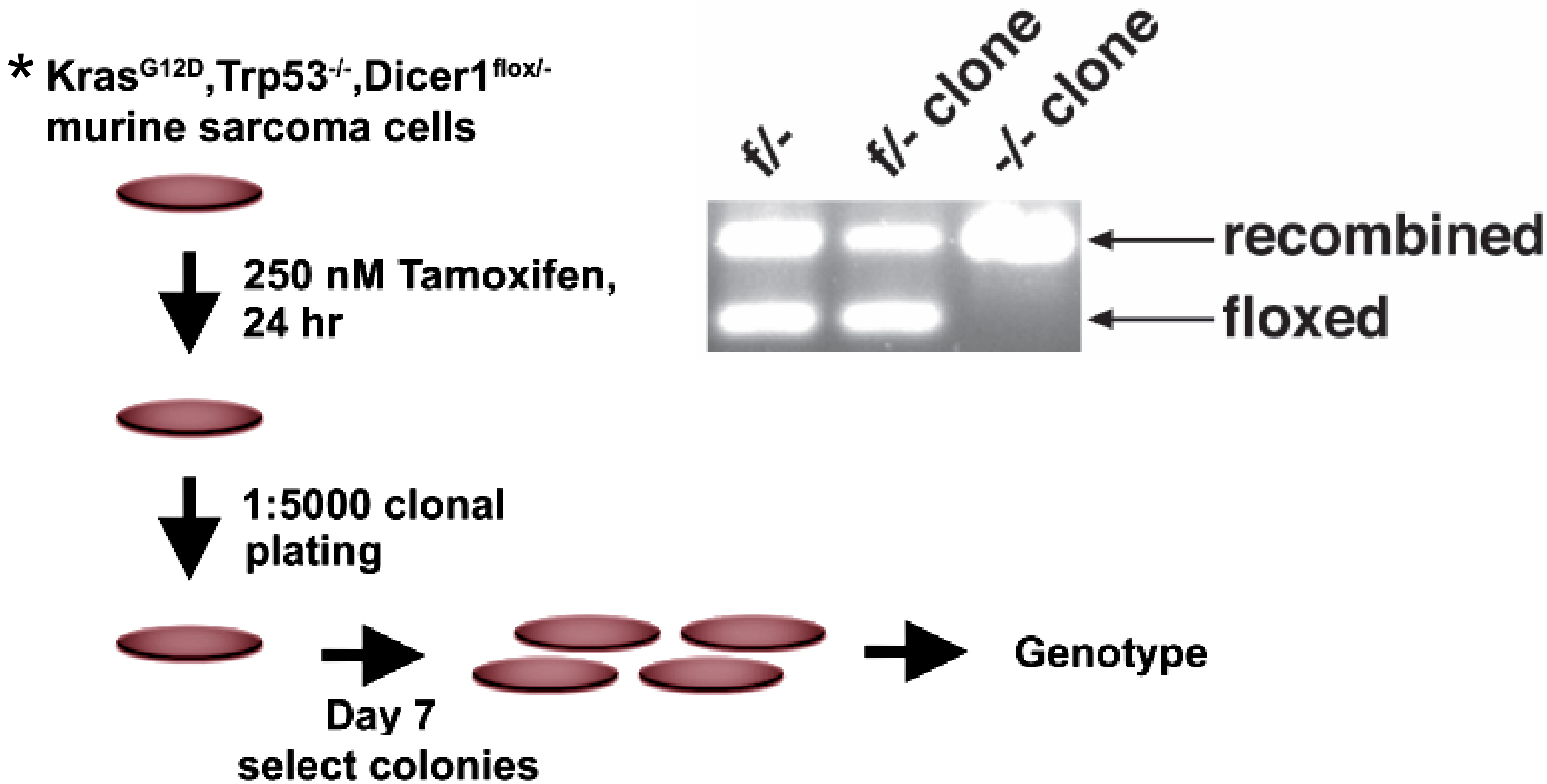


Genes Dev. 2005 Feb 15;19(4):489-501



"However, DCR-/- cells did not contribute to the generation of chimeric mice when injected into blastocysts, unlike DCRneo/+ and DCRneo/neo cells, which gave rise to high-percentage chimeras (data not shown). Furthermore, the Dicer-deficient cells failed to generate detectable teratomas upon subcutaneous injection into nude mice (Fig. 2C)."

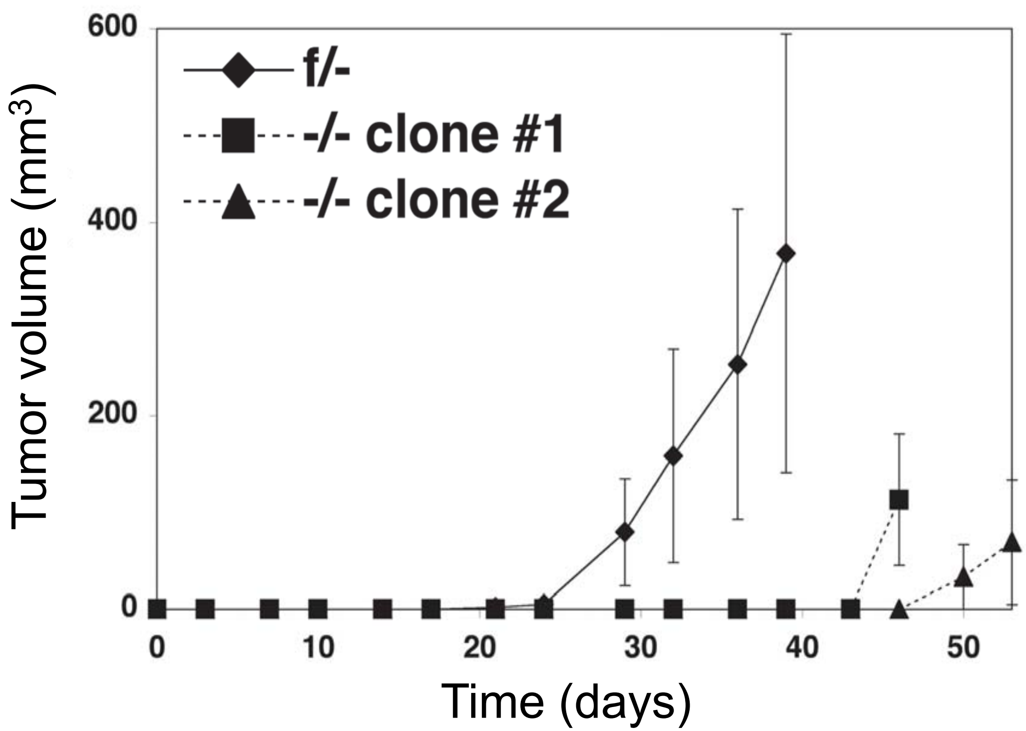
Generation of Dicer null sarcoma cell line

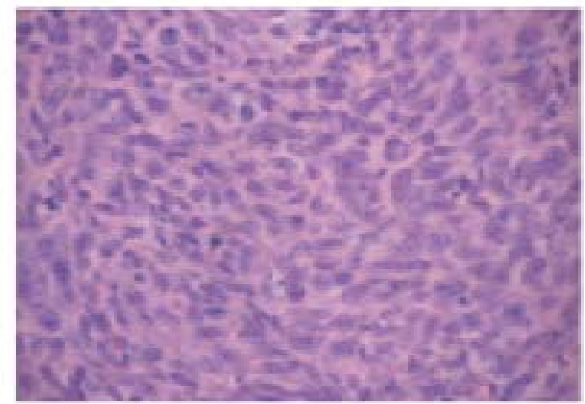


*Generated by Madhu Kumar and Tyler Jacks

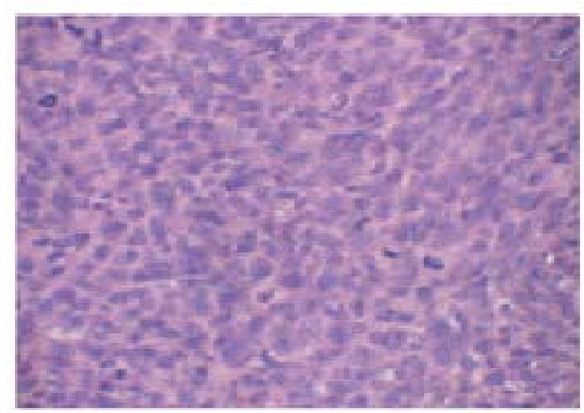
Dicer null cells retain the ability to form tumors

Subcutaneous tumors in nude mice





Dicer1flox/-



Dicer1-/-

Literature on microRNA and Stress

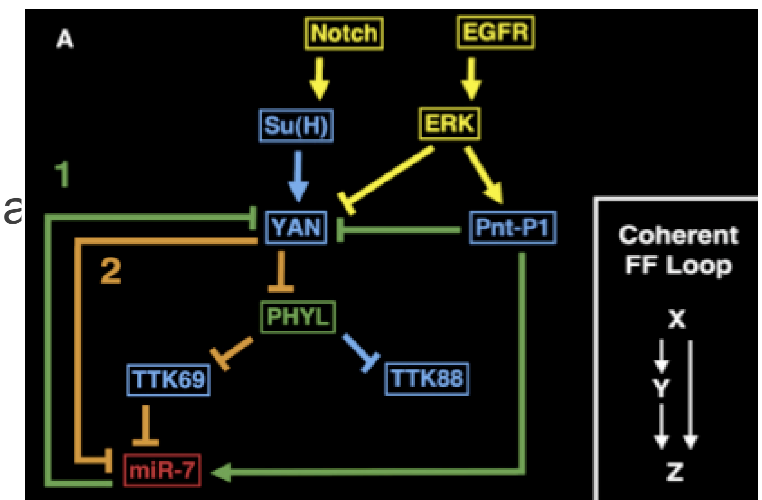
Genetic Data

Knockout of miR-14 in Drosophila results in sensitivity to salt stress and reduced lifespan (Xu et al., Curr Biol 2003)

Heart-specific miR-208 regulates stress-dependent cardiac growth in mice (Rooij et al., Science 2007)

miR-8 regulates the response to osmotic stress in zebrafish embryos (Flynt et al., JCB 2009)

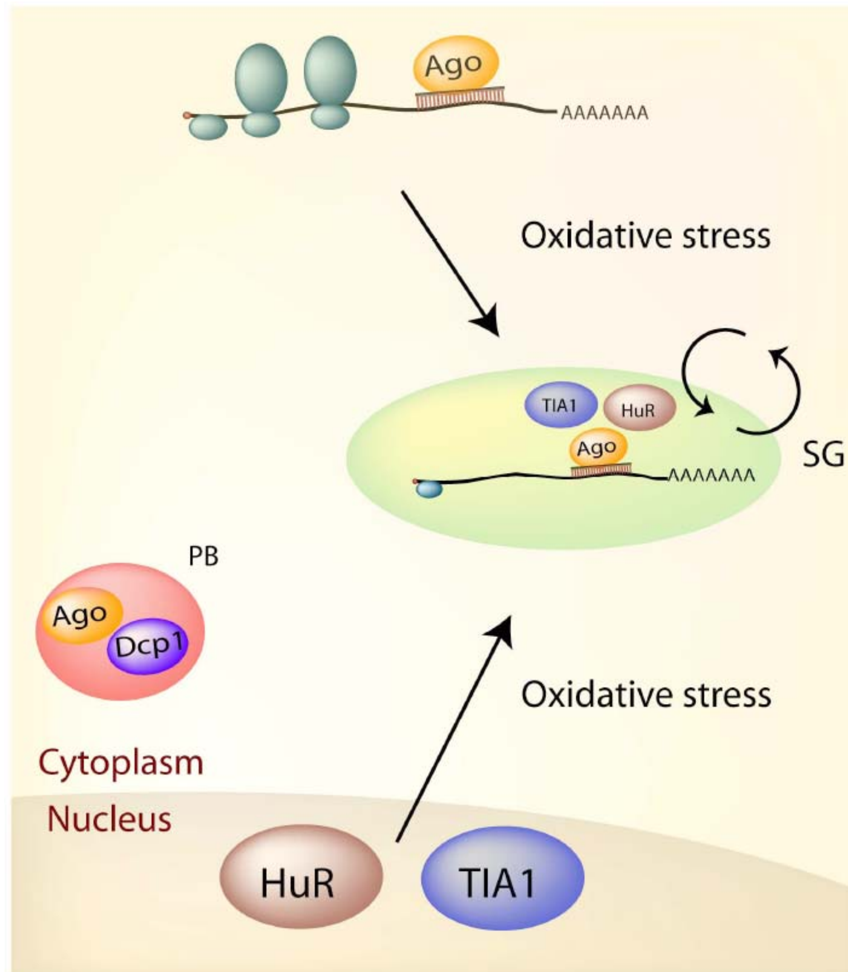
miR-7 stabilizes gene regulatory networks aga
environmental fluctuations during Drosophila
development (Li et al., Cell 2009)



Literature on microRNA and Stress

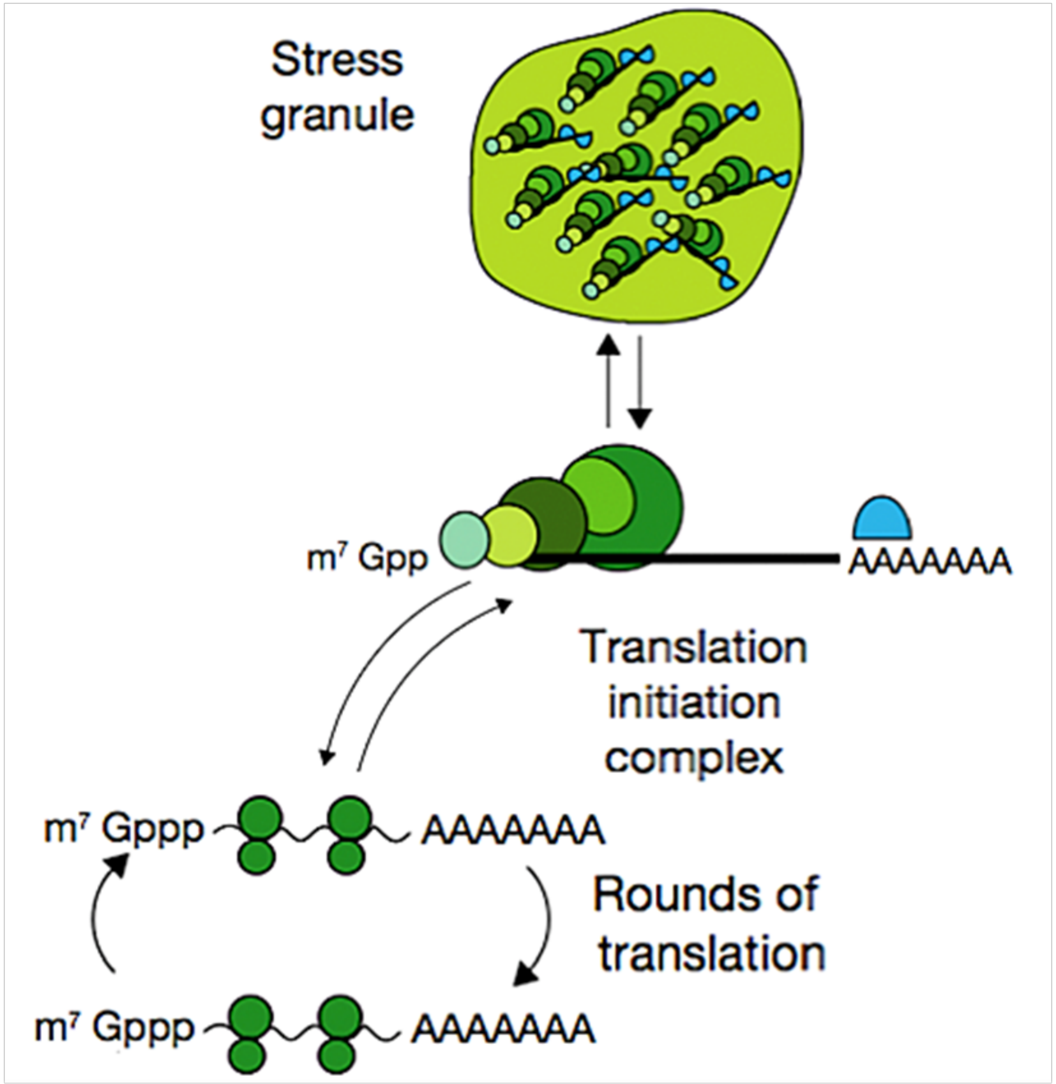
Localization

Upon oxidative stress or inhibition of translation initiation, we found that Argonaute localizes to Stress Granules (SGs) in a miRNA-dependent manner.



Argonaute at SGs continues to exchange with the cytoplasm, in which 50% of Argonaute at SGs are replaced within 20 seconds.
(Leung et al., PNAS 2006)

Stressed cells form cytoplasmic stress granules (SGs) in which mRNA translation is arrested in pre-initiation complex



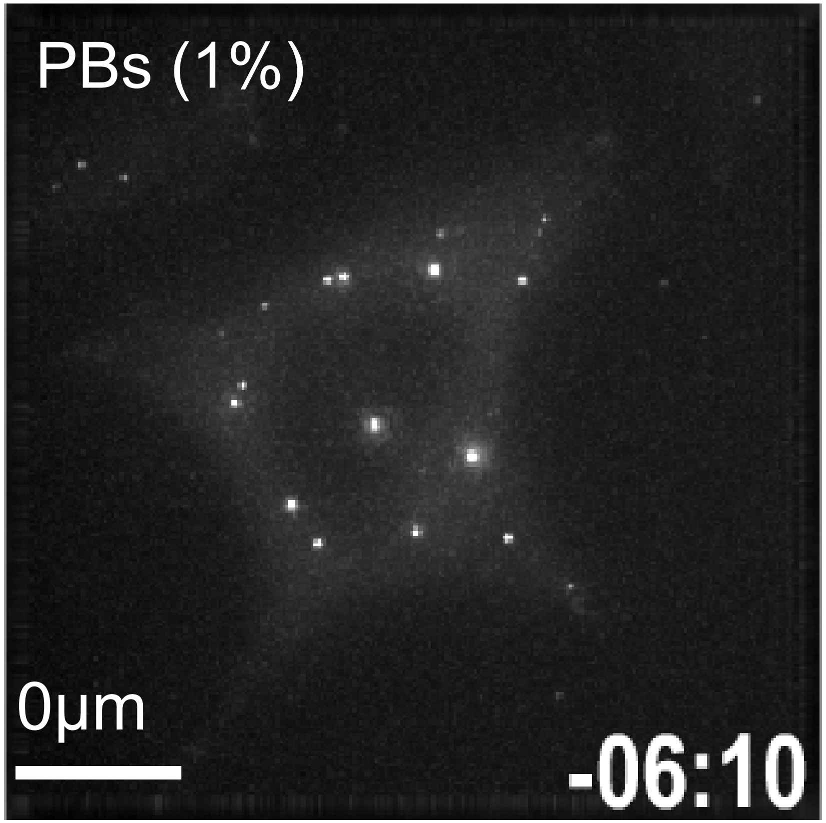
Stress Granules (SGs)

- first identified in plant
- conserved from worm to human
- stalled initiation complex
- enriched with RNA binding proteins (TIA-1, G3BP-1, PABP-1, and Ago2)
- proposed role of mRNA triage (degradation, reinitiation and storage)

modified from
Hilliker and Parker
Nat Cell Biol 2008

Dynamic localization of post-transcriptional regulators

microRNA-binding factor Argonaute (Ago2)



EGFP-Ago2

Time 0: P-bodies with Ago2 - degradative sites for mRNAs

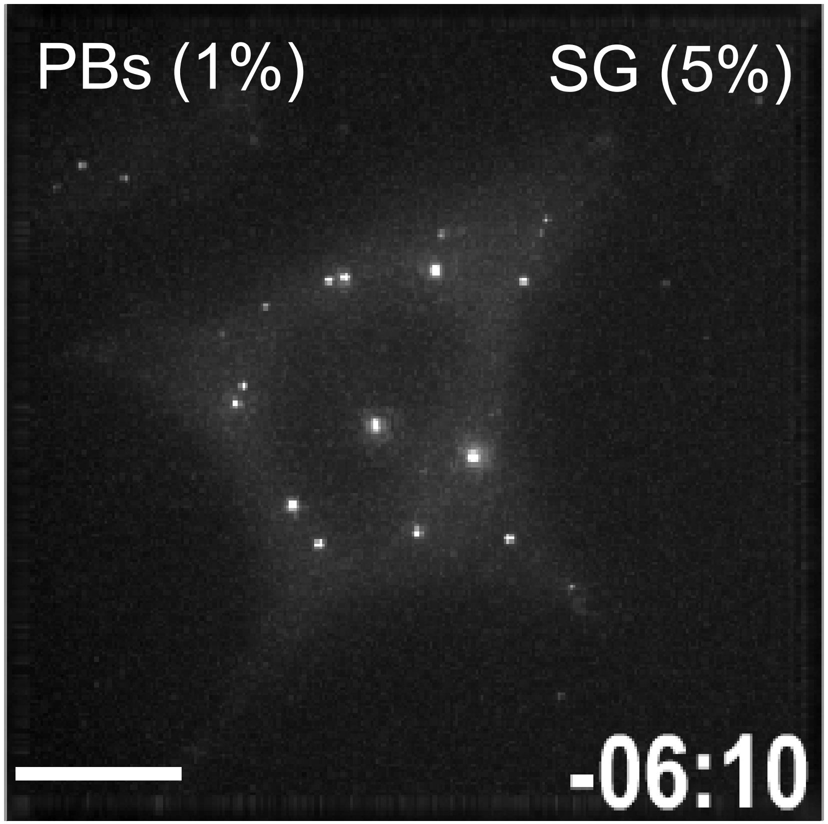
Stress granules forms upon

- inhibition of translation initiation
- heat shock
- UV irradiation
- viral infection
- energy depletion
- proteasomeinhibition
- hypoglycemia
- hypoxia

Leung et al., PNAS 2006

Dynamic localization of post-transcriptional regulators

microRNA-binding factor Argonaute (Ago2)



EGFP-Ago2

Time 0: P-bodies with Ago2 - degradative sites for mRNAs

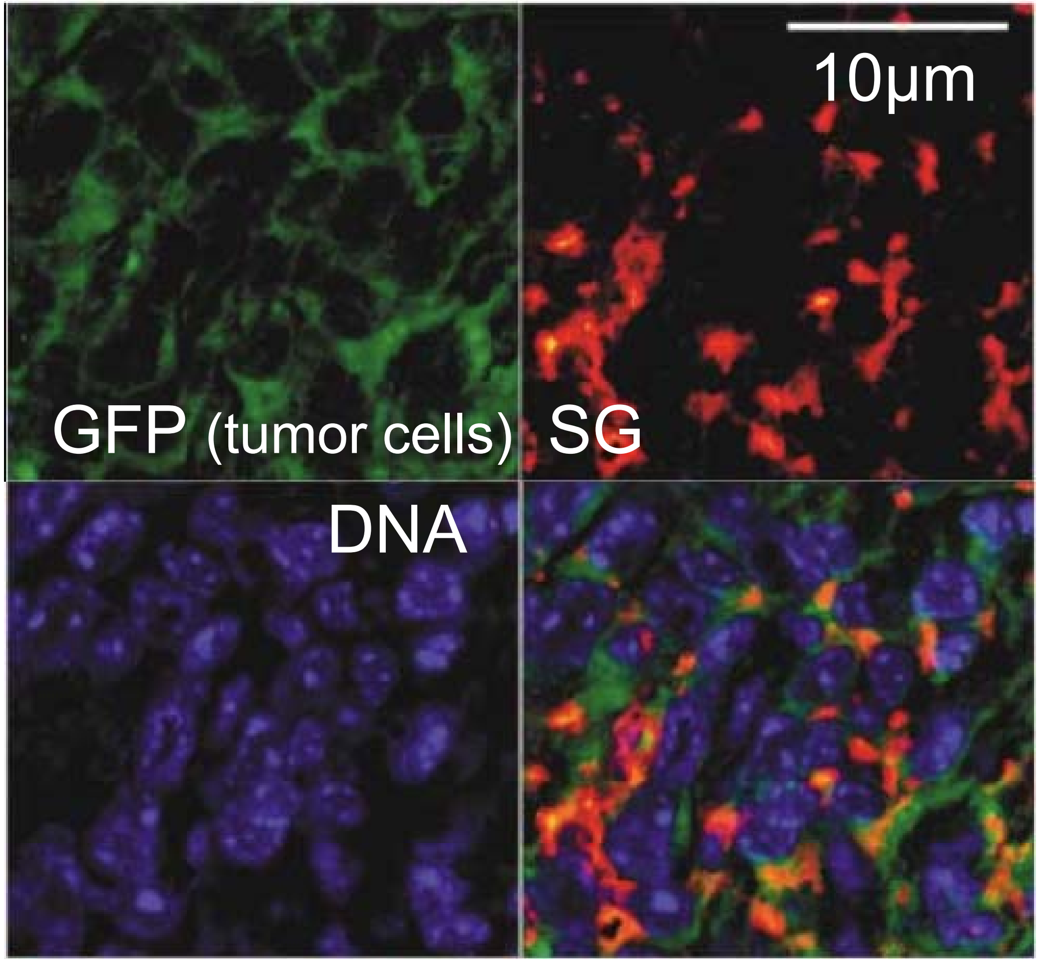
Time 35: Formation of stress granules containing Ago2

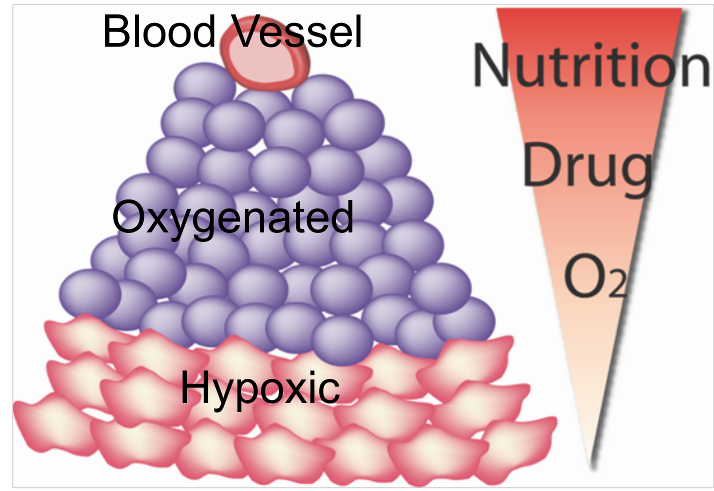
Stress granules forms upon

inhibition of translation initiation
heat shock
UV irradiation
viral infection
energy depletion
proteasomeinhibition
hypoglycemia
hypoxia

Leung et al., PNAS 2006

Stress Granules present in Hypoxic tumor cells

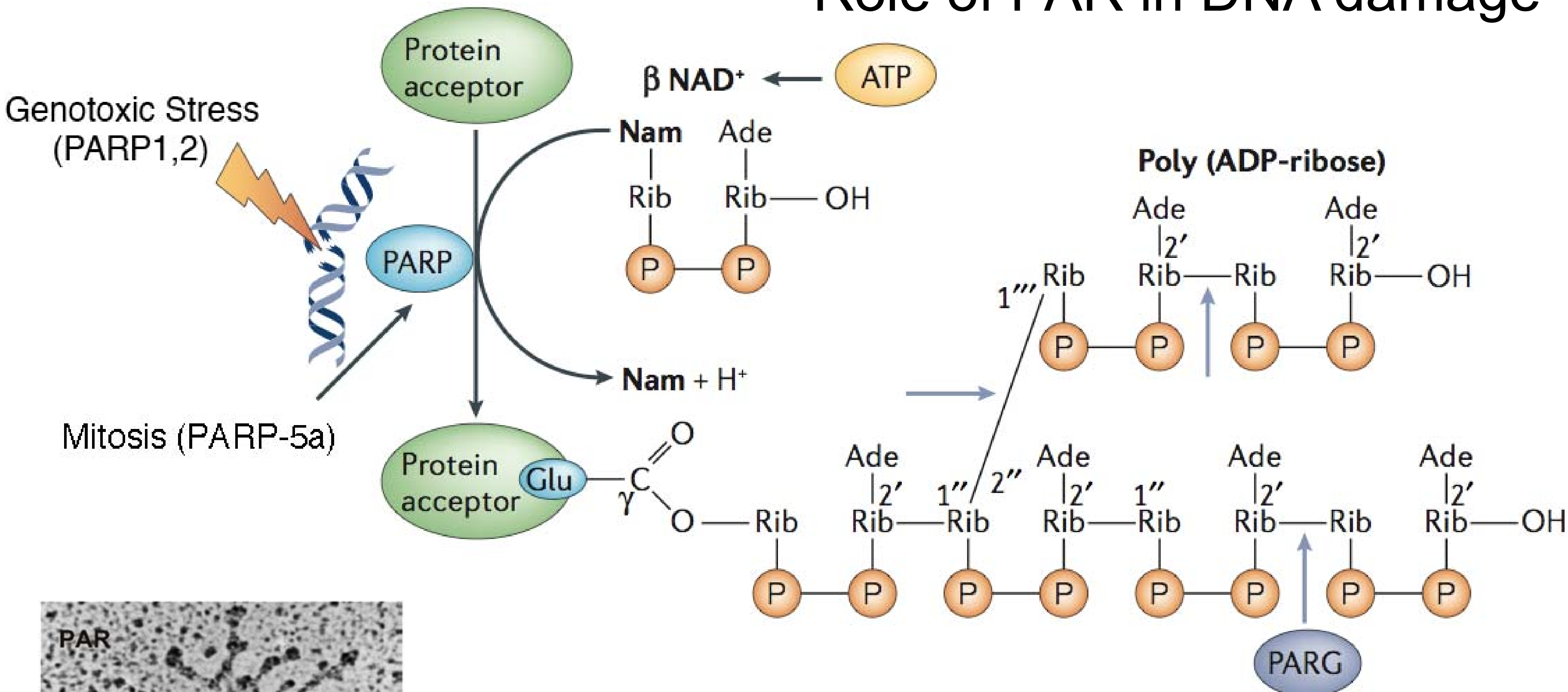


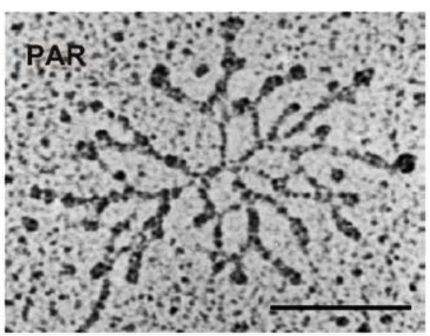


Moeller et al., Cancer Cell 2004

Evidence that Poly(ADP-ribose), PAR, is matrix for SG formation and influences miRNA regulation of translation

Role of PAR in DNA damage





Poly-ADP-ribose (PAR) modified

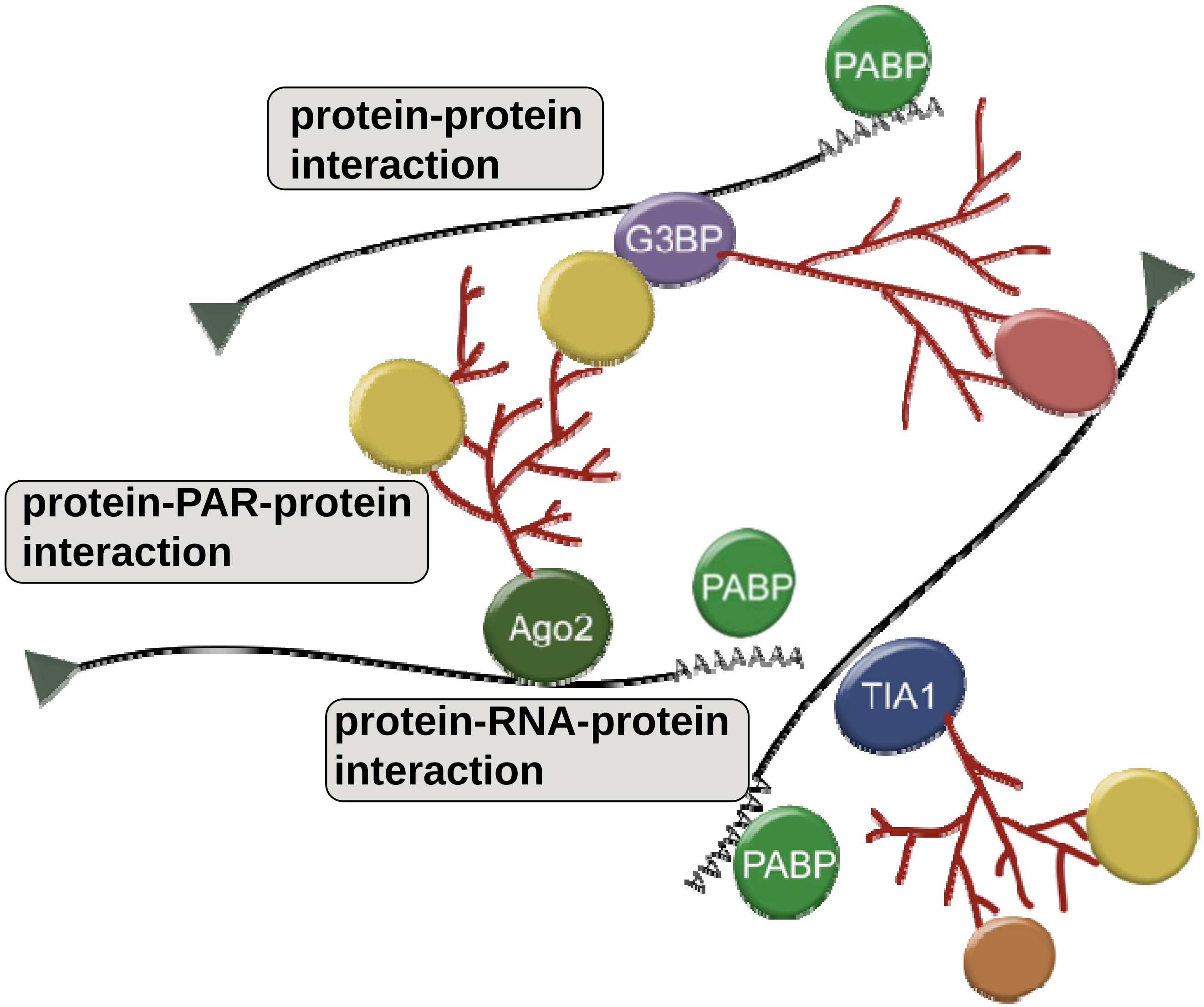
- upon DNA damage
- during mitosis at mitotic spindle

modified from Schreiber et al
Nat Rev Mol Cell Biol 2006

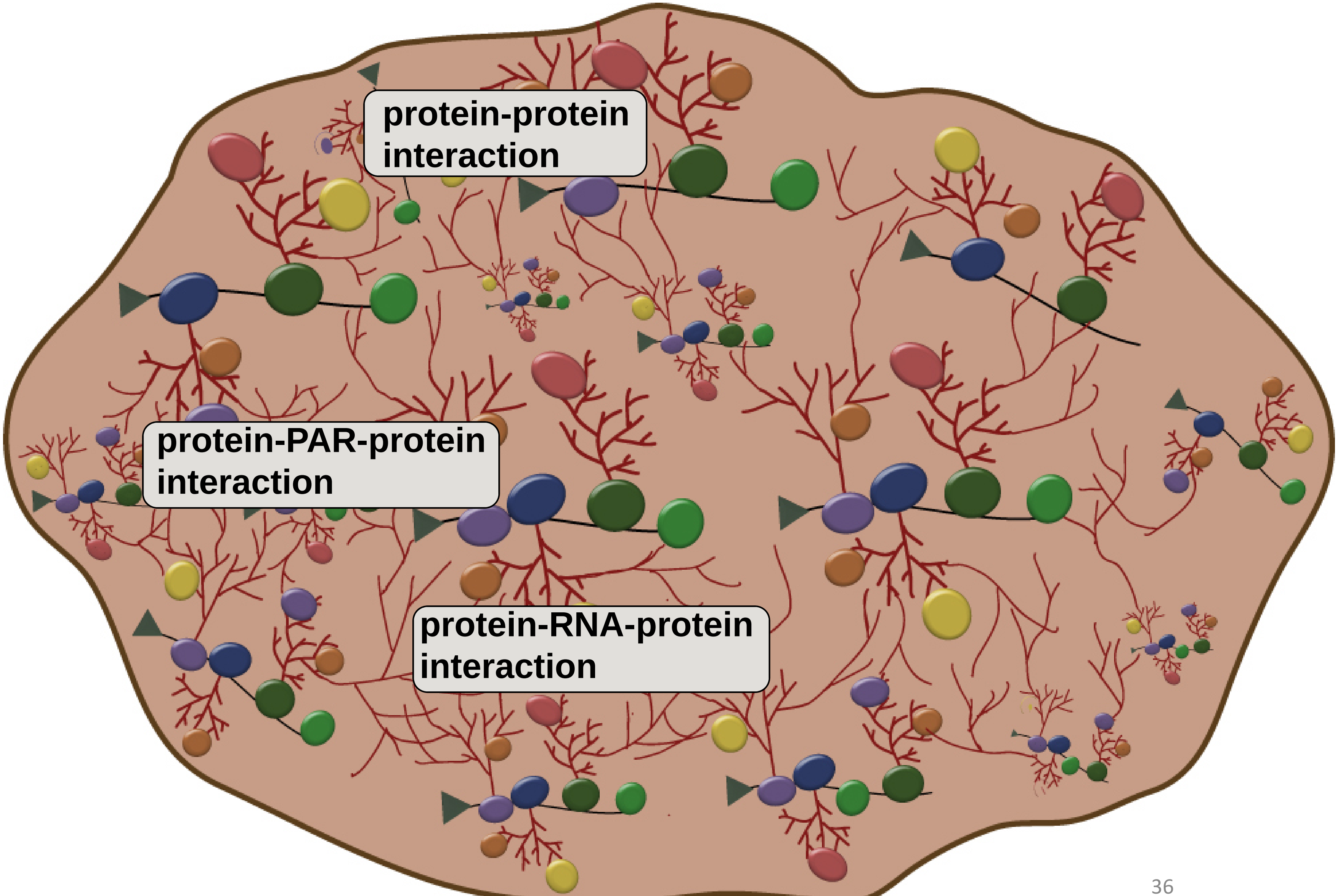
Poly(ADP-ribose) is **essential** in multicellular eukaryotes

- Required for Chromosome Integrity, Transcription and Cell Division
- PARP-1 is targeted by pharmaceutical companies for BRCA1- Cancer
- Inhibitors have been beneficial in ischemia, inflammation, degenerative and vascular diseases

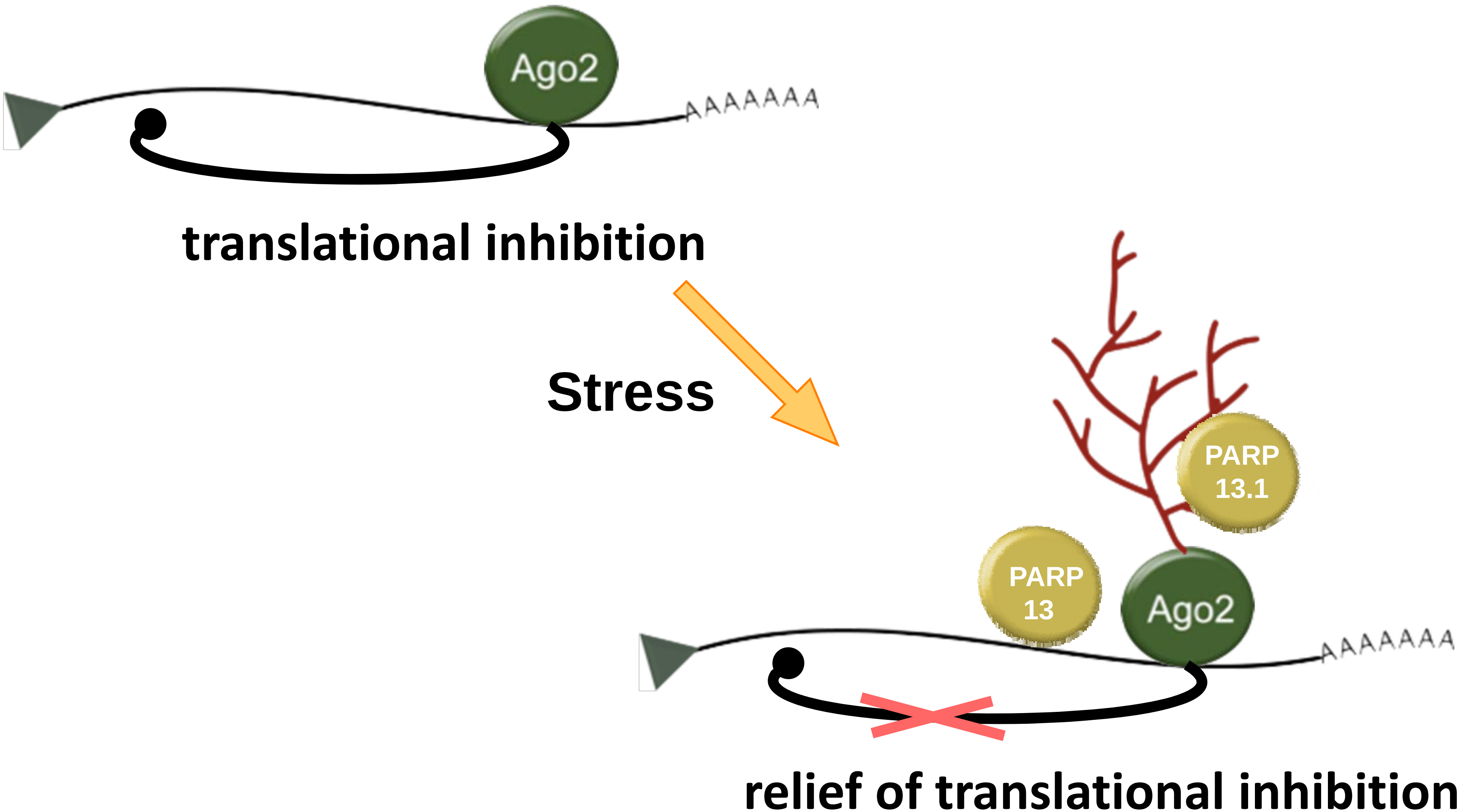
Proposed model of Stress Granule formation



Proposed model of Stress Granule formation



Proposed model of gene regulation by Poly(ADP-ribose)



Conclusions

- PAR, 5 PARPs and 2 PARG isoforms are localized in RNA-binding protein enriched-Stress Granules.
- Poly(ADP-ribose) is required for structural integrity of Stress Granules.
- Specific RNA-binding proteins are modified by PAR upon stress, effecting post-transcriptional gene regulation.
- Under stress conditions, the level of miRNA regulation is reduced.
- Poly(ADP-ribose) probably regulates translation under normal and stress conditions.

A role of microRNA regulation-Robustness

1) Stabilize quiescent and differentiated state
   - microRNA-let7 suppresses Ras, etc (growth) (*Frank Slack)*
   - Cell-type specific microRNAs suppresses genes expressed in other cellular states (*Lee Lim-05 and Stephen M. Cohen-05*)
   - Suppress cell-type specific genes
2) Control responses to changes in environment
   - Protection against abnormal cellular states (p53 and NF-κB) and stabilize cellular states from transient “stress”

Acknowledgments

Sharp Lab, MIT
Arvind Ravi
Joel Neilson
Grace Zheng
Anthony Leung
Margaret Ebert
Amanda Young
Mary Lindstrom
Allan Gurtan

Tyler Jacks
Madhu Kumar

Paul Chang
SejalVyas

Christopher Burge
Rickard Sandberg
Arup Sarma

Rudolf Jaenisch

Paul Anderson (HMS)
Nancy Kedersha

Rod Bronson (Tufts)

KOCH INSTITUTE
for Integrative Cancer Research at MIT

Thank you for the opportunity to present this lecture